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Tissue Distribution of Enrofloxacin in African Clawed Frogs (*Xenopus laevis*) after Intramuscular and Subcutaneous Administration

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As part of an enrofloxacin pharmacokinetic study, concentrations of enrofloxacin and ciprofloxacin (metabolite) were measured in various tissues (brain, heart, kidney, liver, lung, and spleen) collected from treated (subcutaneous delivery, *n* = 3; intramuscular delivery, *n* = 3; untreated controls, *n* = 2) adult female *Xenopus laevis* by using HPLC. Enrofloxacin was rapidly absorbed after administration by either route and readily diffused into all sampled tissues. Enrofloxacin and ciprofloxacin were present in the tissue samples collected at 8 h. The highest average tissue concentrations for enrofloxacin were found in kidney, with the lowest concentrations in liver. Ciprofloxacin tissue concentrations paralleled but were always lower than those of enrofloxacin for all time points and tissues except brain and kidney. These results, together with previously published pharmacokinetic data and known minimal inhibitory concentrations of common pathogenic bacteria, provide a strong evidence-based rationale for choosing enrofloxacin to treat infectious diseases in *X. laevis*.

Many aquatic frog pathogens are considered susceptible to fluoroquinolones; therefore, enrofloxacin is a logical drug of choice.[1,6,11] Our lab recently used HPLC to determine the plasma pharmacokinetics of enrofloxacin (single 10-mg/kg dose; Baytril, Bayer HealthCare, Monheim, Germany) after subcutaneous and intramuscular administration in *Xenopus laevis*.[7] The results showed that *Xenopus* metabolize enrofloxacin in a manner similar to that of mammals. The plasma pharmacokinetics and the wide spectrum of activity for enrofloxacin and its active metabolite ciprofloxacin indicate that enrofloxacin is a safe and efficacious antibiotic choice for *X. laevis*. Whereas enrofloxacin has been shown to penetrate most tissues and achieve therapeutically effective concentrations in mammals,[3,5,13,15] this information remained unknown for *Xenopus*. Here we sought to determine whether enrofloxacin disseminated from plasma and concentrated in various tissues of *Xenopus*.

Materials and Methods

Animals. We used 8 physically healthy, adult female frogs (*X. laevis*; weight, 112 to 175 g) for the tissue distribution portion of this IACUC-approved project. The frogs were fed a commercial diet (Frog Brittle, Nasco, Fort Atkinson, WI) and housed in an AAALAC-accredited facility in a 300-L pond-style holding tank at a stocking density of approximately 1 frog per liter. The following water parameters were maintained: temperature, 16 to 21 °C; pH, 7.0 to 8.5; average hardness, 15 to 30 dGH; total chlorine, less than 0.01 mg/L; chloramines, less than 0.01 mg/L; ammonia, less than 0.25 mg/L; nitrite, less than 0.20 mg/L; nitrate, 0.00 to 50.0 mg/L; copper, less than 0.02 g/L; water fecal coliform count, less than 2000 organisms per 100 mL; conductivity, 100 to 300 μΩ, and dissolved oxygen, 8.00 to 9.00 mg/L. The room was on a 12:12-h light:dark cycle.

Study design. Frogs were assigned to 1 of 4 groups: subcutaneously administered enrofloxacin, *n* = 3; intramuscularly administered enrofloxacin, *n* = 3; subcutaneously administered saline, *n* = 1; and intramuscularly administered saline, *n* = 1. Each frog was housed individually after injection.

Tissue collection. Approximately 5 min prior to scheduled tissue collection time points (1, 4, and 8 h after injection for enrofloxacin-treated frogs; 1 h after injection for saline-treated frogs), frogs were submerged in tricaine methanesulfonate (5 g/L and buffered with 10 g sodium bicarbonate) until both righting and toe-pinch reflexes were absent. The deeply anesthetized frogs underwent cardiac venipuncture followed by euthanasia via cardiac removal. Samples of brain, heart, kidney, liver, lung, and spleen were collected and placed in a −80 °C freezer until concentration analyses were performed.

Tissue distribution analysis. HPLC was used to determine the concentrations of enrofloxacin and its metabolite ciprofloxacin in sampled tissue. Assays were performed as done in a previous study using *X. laevis*.[9] The limit of quantification was 0.05 μg/mL, which was the lowest level that gave a linear response on our calibration curve. Tissue concentrations then were compared with previously determined plasma concentrations[9] to derive tissue:plasma concentration ratios.

Statistical analysis. Excel 2007 (Office, Microsoft, Redmond, WA) was used to compute average tissue concentration and associated values. The threshold used to determine statistical significance was a *P* value of less than 0.05.

Results

The concentrations of enrofloxacin and ciprofloxacin in tissue samples from enrofloxacin-treated frogs are shown in Table 1; tissue levels of enrofloxacin and ciprofloxacin from saline-treated frogs (controls) were 0 μg/g and are not included in the table. After both subcutaneous and intramuscular delivery,

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Table 1. Tissue concentrations (μg/gm) of enrofloxacin (E) and ciprofloxacin (C) and tissue:plasma ratios of enrofloxacin (T_E:P_E), ciprofloxacin (T_C:P_C), and enrofloxacin plus ciprofloxacin (T_{E+C}/P_{E+C}) in *X. laevis* (n = 6) at various times after a single dose of enrofloxacin (10 mg/kg IM or SC)

	Brain					Heart					Kidney				
	E	C	T_E:P_E	T_C:P_C	T_{E+C}:P_{E+C}	E	C	T_E:P_E	T_C:P_C	T_{E+C}:P_{E+C}	E	C	T_E:P_E	T_C:P_C	T_{E+C}:P_{E+C}
IM, 1 h	5.2	1.4	0.9	5.7	1.1	3.6	0.9	0.61	3.9	0.7	32.7	1.6	5.5	6.5	5.5
IM, 4 h	4.3	1.9	2.4	2.1	2.3	0	0.9	0	1	0.3	0	0.3	0.3	0	0.1
IM, 8 h	1.1	1.7	0.6	8.3	2.6	1.2	1.1	0.65	5.6	2.1	8.40	2	4.47	10.05	9.6
SC, 1 h	3.3	1.9	1.2	9.0	1.8	13.1	1.4	4.79	6.5	4.9	24	1.1	8.79	5.38	8.5
SC, 4 h	1.9	1.6	1.1	2.8	1.53	7.4	1.3	4.43	2.3	3.9	8.20	1.1	4.94	2.02	4.2
SC, 8 h	0.8	1.5	0.5	1.0	0.7	1.3	0.4	0.85	0.3	0.6	2.96	0.5	1.86	0.3	1.1

	Liver					Lung					Spleen				
	E	C	T_E:P_E	T_C:P_C	T_{E+C}:P_{E+C}	E	C	T_E:P_E	T_C:P_C	T_{E+C}:P_{E+C}	E	C	T_E:P_E	T_C:P_C	T_{E+C}:P_{E+C}
IM, 1 h	0.1	0.9	0.0	3.8	0.2	3.5	0.4	0.58	1.5	0.6	4.40	1	0.74	4.33	0.9
IM, 4 h	0.4	0.7	0.2	0.8	0.4	4.3	0.5	2.36	0.5	1.8	7.91	1.2	4.34	1.4	3.4
IM, 8 h	0.7	0.7	0.4	3.4	1.3	2.1	0.4	1.13	1.8	2.3	ND	ND	ND	ND	ND
SC, 1 h	0.4	0.5	0.1	2.3	0.3	2.7	0.2	0.98	1.1	1.0	18	1	6.61	4.95	6.5
SC, 4 h	0.7	0.4	0.4	0.8	0.5	2.5	0.2	1.52	0.4	1.2	8.89	0.4	5.36	0.79	4.2
SC, 8 h	0.4	1.1	0.2	0.7	0.5	1.3	0.2	0.8	0.1	0.5	4.4	0.7	2.77	0.42	1.7

ND, not determined.

Table 2. Minimal inhibitory concentrations (μg/mL) of enrofloxacin and ciprofloxacin in various aquatic pathogens

	Enrofloxacin	Ciprofloxacin
Escherichia coli	0.004–0.015[a]	≤0.5[b]
Aeromonas. salmonicida salmonicida	0.008–0.03[a]	0.015–1.0[c]
Aeromonas hydrophila	0.25[d]	0.00375–1.0[e]

[a]From reference 7
[b]From reference 3
[c]From reference 4
[d]From reference 6
[e]From reference 12

enrofloxacin was rapidly absorbed and readily diffused into all sampled tissues (brain, heart, kidney, liver, lung, and spleen). Enrofloxacin and its metabolite ciprofloxacin were present in the tissue samples collected at 8 h. The average kidney concentration of enrofloxacin in kidney (14.66 μg/g) was significantly (t test, P = 0.04) higher than that in liver (0.46 μg/g). Ciprofloxacin concentrations paralleled those of enrofloxacin for all time points in all tissues except brain and kidney.

Discussion

Enrofloxacin was rapidly absorbed after both intramuscular and subcutaneous administration to *X. laevis* frogs and diffused into all tissues studied. Tissue ciprofloxacin concentrations rose rapidly after the administration of enrofloxacin and remained above the detection limit for at least 8 h. The organ of metabolism of fluoroquinolones has not been determined for amphibians. However, kidney showed the highest concentrations of enrofloxacin in the current study, suggesting that renal metabolism may be the preferred route. By contrast, the enrofloxacin concentrations in liver were low at all time points, indicating that the liver may not serve as a major site of metabolism. This pattern is distinctly different from that in mammals, in which liver metabolism and intestinal excretion are the major routes of elimination. The current study shows that enrofloxacin is well absorbed and distributed into tissues. Its metabolite ciprofloxacin, which similarly appeared in high concentrations in various tissues, is also active as an antimicrobial compound. Comparisons of the activity of each drug show that, in general, enrofloxacin is more active against gram-positive bacteria, whereas ciprofloxacin is more active against gram-negative bacteria.[2,7] Therefore, when the parent compound is administered, its metabolite produces an additive effect that increases activity, especially against gram-negative bacteria.

The enrofloxacin concentrations in kidney and heart at the 4-h time point of intramuscularly treated frogs were essentially zero (Table 1). This finding is perplexing, particularly when one considers the high enrofloxacin concentrations achieved at 1 and 8 h. This variation in the results is not easily explained but is inherent when measuring tissue concentrations from small samples in animals. The aberrant data probably are either artifacts or outliers that cannot be verified or refuted without further investigation.

Although pharmacokinetic analysis could not be performed due to the limited time points and tissues sampled in the current study, the results show that enrofloxacin and ciprofloxacin diffused from the plasma and concentrated in the tissues at levels above the minimal inhibitory concentrations of various aquatic pathogenic bacteria (Table 2). The high tissue concentrations are evidence that these drugs penetrate tissue and that there is no barrier to diffusion into tissues. However, plasma concentrations typically are used to predict the therapeutic efficacy of compounds in this class of drugs.[10,12] In contrast, the authors of a previous study[10] concluded that the antibiotic concentrations in the interstitial fluid at the target site are responsible for the antibacterial effect and are more relevant in predicting therapeutic efficacy than are plasma concentrations. The homogenized

tissue concentrations reported previously[10] may overrepresent those in the extracellular fluid of tissues because enrofloxacin is known to concentrate intracellularly. However, because enrofloxacin is much more lipophilic than is ciprofloxacin (by approximately 100-fold[1,14]), the tissue enrofloxacin concentrations we report here may be underestimates.

Despite these limitations in the interpretation of tissue concentrations due to small sample size and inherent variability, the current study shows that there are no barriers to diffusion of enrofloxacin into the tissues examined. Our results also show that kidney concentrations of enrofloxacin were significantly higher than were those in liver, suggesting that the kidney rather than the liver (as seen in many mammalian species) may be the major route of metabolism. Collectively, these current findings regarding tissue distribution, together with previously published data from pharmacokinetics studies and the known minimal inhibitory concentrations of enrofloxacin in aquatic pathogenic bacteria, provide a strong evidence-based rationale for choosing enrofloxacin to treat infectious diseases in *X. laevis*.

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